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14. ABSTRACT A novel exposure system operating in the W-band (75-110 GHz) was designed, characterized and used for identifying millimeter wave (MMW) exposure parameters (frequency, modulation schemes and electric field magnitude) that could potentially accelerate recovery from fatigue in skeletal muscle. The system designed allows electrical stimulation of muscle from mouse toe (<i>flexor digitorum brevis</i> (FDB)) and measurement of contractile force during MMW exposure within an organ bath under conditions where the temperature of the muscle is maintained at the physiologically relevant value of $35 \pm 0.2^\circ$ C. Design and characterization of the exposure system were performed using the finite-difference time-domain numerical method and experimental measurements. The design, characterization, and testing as well as experimental results are presented in this report.					
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FINAL PERFORMANCE REPORT

Project Title: Millimeter wave-based fatigue countermeasure research for improving performance and prolonging combat effectiveness of warfighters

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Abstract

A novel exposure system operating in the W-band (75-110 GHz) was designed, characterized and used for identifying millimeter wave (MMW) exposure parameters (frequency, modulation schemes and electric field magnitude) that could potentially accelerate recovery from fatigue in skeletal muscle. The system designed allows electrical stimulation of muscle from mouse toe (*flexor digitorum brevis* (FDB)) and measurement of contractile force during MMW exposure within an organ bath under conditions where the temperature of the muscle is maintained at the physiologically relevant value of $35 \pm 0.2^\circ \text{C}$. Design and characterization of the exposure system were performed using the finite-difference time-domain numerical method and experimental measurements. The design, characterization, and testing as well as experimental results are presented in this report.

Introduction

MMW radiation in W-band, specifically 94 GHz, is used in military radar and in non-lethal weapon systems where it causes an intense sensation of heat on the skin [1, 2]. While such thermal effects of MMW radiation in W-band are well-known, a number of studies suggest that MMW radiation can elicit bioeffects that cannot be explained based on temperature increases during exposure. Instead, these bioeffects are non-thermal in nature [3], with some being frequency specific [4-7]. Moreover, in Eastern Europe and Russia, therapeutic applications of MMW radiation attributed primarily to non-thermal effects are widely accepted and being used clinically [3].

Microwave radiation at 2.45 GHz has been reported to induce changes in isolated frog skeletal muscle that could potentially counteract muscle fatigue [8]. Our goal was to determine whether MMW exposure could exert a similar effect on skeletal muscle. This project involved the design, characterization and testing of a W-band MMW exposure system using a strategy developed in our laboratory [9] for assessing non-thermal radiofrequency effects on skeletal muscle. The system incorporates an organ bath to suspend a mammalian, fast-twitch muscle for monitoring contraction elicited by electrical stimulation during exposure. The design, characterization and testing of the MMW exposure system was accomplished using a combination of the numerical Finite-Difference Time-Domain (FDTD) method and experimental measurements. The exposure system has been used to perform a series of carefully planned contractile force measurements on FDB muscle exposed to various types of MMW fields both under fatiguing and non-fatiguing conditions.

Materials, Methods and Design

A. Exposure Chamber

The central feature of the exposure system consists of a custom-designed, double jacketed glass organ bath (Radnoti, LLC, Monrovia, CA) that permits an intact FDB, a fast-twitch muscle obtained from mouse toe averaging 4 mm in length and 0.8 mm in diameter, to be positioned between two waveguides for MMW exposure and also between two parallel platinum electrodes for simultaneous electrical stimulation of

muscle contraction (Fig. 1a). The waveguides are placed diametrically opposite to each other. The muscle is suspended vertically within the inner chamber (25 mm diameter x 70 mm height, 29.5 ml) of the organ bath via a no. 5 silk suture attached to the arm of an Aurora Scientific model 300 servo-motor to register contractions. The distal end of the muscle is held taut by a glass hook at the bottom of the organ bath (Fig. 1b). The two platinum electrodes (diameter = 1 mm) that are used to electrically stimulate the muscle by a pre-programmed protocol via an Aurora Scientific model 701B DC Stimulator at frequencies varying from 1 to 100 Hz are spaced 1 cm apart. LabVIEW programs control the protocols for electrical stimulation and for measuring and recording the generated contractile force.

The muscle is continuously perfused from the bottom of the chamber at a flow rate of 1 ml/min with Tyrode solution [9] and maintained at $35^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ by heated water flowing through the outer jacket of the organ bath. Temperature is monitored by a non-perturbing fluoroptic temperature probe and thermometer (Luxtron model 790 and SFW probes) and recorded by a custom-written LabVIEW program (version 2009). The probe gains entry through a hole in the lid and is positioned directly above the muscle (Fig. 1b).

B. MMW Delivery

Two W-band waveguides enter the inner chamber of the organ bath via side ports that use specially designed glass sleeves and Sylgard (184 silicone elastomer, Dow Corning Corp.) sealant to allow for precise positioning of the waveguides without allowing leaks. The waveguides are carefully positioned facing one another with the muscle located between them. Rapid attenuation of the electric (E) field in the conductive Tyrode solution at MMW frequencies requires extremely close placement of each waveguide to the muscle. The minimum spacing that does not allow contact between the muscle and waveguides during muscle contractions is 0.2 mm. Thus, the waveguides are placed 0.48 mm apart using adjustable micrometer stages. A magnifying camera (Edmund Optics model EO-0312C) inserted into a side port of the organ bath aids in aligning the waveguides with respect to the muscle and also permits video recording of muscle contractions throughout an experiment. The waveguides can be oriented with the E field of the TE_{10} mode parallel to either the muscle length or width. Each waveguide is connected to a signal generator (Hewlett Packard 83640A synthesized sweeper), multiplier (HAFMW6-182, HX1), amplifier (either HHPAW-262, HXI, rated at 250 mW or Quinstar QPN-94043030-C2, rated at 1W), isolator and directional coupler. The schematic and photograph of the MMW instrumentation as well as the tapered matching element and photograph of the assembled matching element are shown in Fig. 2.

Custom-written LabVIEW programs control all MMW source parameters (e.g amplitude, frequency, pulse modulation), and monitor the power into the waveguides. The return loss at the open end of the waveguides terminated in Tyrode solution was measured using a network analyzer (Agilent E8364B, Agilent N5260A controller and OML V10VNA2-T/R MMW modules) and found to be 1.7 dB indicating that almost 67% of the power was reflected back into the waveguides. To reduce reflections at the interface of the open end of the waveguides and the Tyrode solution, each waveguide is terminated with a gold-plated tapered dielectric (Emerson & Cuming, Eccostock HiK 4) matching element (Fig. 2b and 2c). This tapered dielectric element achieves an impedance match by gradually transitioning the waveguide impedance to match the

impedance of the Tyrode solution [10]. The resulting return loss with the matching element installed was 11 dB.

C. FDTD Simulations

To obtain the detailed E field distribution in the region of the organ bath containing the muscle, the SolidWorks model of the organ bath (Fig. 1) was imported into the commercially available FDTD software package SEMCAD (SPEAG, Zurich, Switzerland, version 14.4.0). The dielectric properties of the materials used in the SEMCAD simulation (Table 1) were obtained either from literature [11] or from measurement of the reflection coefficient using a network analyzer (Agilent E8364B, Agilent N5260A controller and OML V10VNA2-T/R MMW modules) and an open-ended coaxial dielectric probe kit. Fig. 3 shows the 3D FDTD mesh of the region containing the waveguides, matching elements, stimulating platinum electrodes and the muscle placed in Tyrode solution. The base mesh size was set to 0.16 mm, however, the muscle was meshed with a much smaller mesh size (0.01 mm) using the adaptive meshing feature in SEMCAD. The computation was performed using one GPU hardware accelerator (nVIDIA Tesla C2050). The simulation time was 4 hours and the computational speed was 190 MCells/s.

D. Experiments

Temperature stability of the Tyrode solution (increase of no more than 0.2°C from the physiologically relevant value of 35 °C) was verified for all MMW exposures by first measuring the temperature of the Tyrode solution with a non-perturbing fluoroptic probe placed at the location of the muscle (muscle absent). The probe was placed as close to the muscle as possible during MMW exposure experiments.

In the first series of experiments, the muscle was subjected to a fatigue protocol by electrically stimulating it with 60 Hz pulses (pulse width 0.5 ms) delivered every four seconds for a total period of two minutes to elicit a rapid decline in force production, followed by a rest period of 40 minutes. This series of stimulations was repeated a total of four times on the same muscle, the first two times serving as control and the next two for establishing the effect of the MMW exposure.

In the second series of experiments, 60 Hz stimulations were applied to the muscle every five minutes for 180 minutes to obtain a series of reproducible contractile responses. The responses during the first 20-minute period established a stable baseline. Then MMW fields were applied for 25 minutes, followed by a 15-minute rest period. This pattern was repeated a total of four times over the period of 180 minutes.

TABLE 1
DIELECTRIC PROPERTIES AT 94 GHz

	ϵ_r	σ (S/m)
Tyrode solution	9.8	57
Skeletal Muscle	9*	50*
Matching element	3.4	0.6

* Obtained from [10]

TABLE 2
PERCENT OF MUSCLE EXPOSED TO THE ELECTRIC FIELD

Input Power (W)	Electric Field Magnitude (kV/m)			
	> 0.25	> 0.5	> 1	> 2
0.25 and 0.25	53.7%	19.4%	2.3%	0%
0.25 and 1	62.7%	41.4%	11.3%	1.5%

Results

Characterization and testing

Fig. 4a illustrates the E field distribution when the input power to each of the waveguides is 250 mW, the directions of the TE₁₀ mode E fields in the two waveguides are perpendicular to each other, and the signal sources are in phase and 180° out of phase, respectively. This orientation of the waveguide TE₁₀ mode fields was chosen because simulation results showed that, for parallel TE₁₀ mode fields, a region of null E field exists in the region containing the muscle. Fig. 4b illustrates the E field distributions for a waveguide input power of 1W on one side and 250 mW on the other side (only one 1W amplifier was available in our laboratory), when the directions of the TE₁₀ mode E fields in the two waveguides are perpendicular to each other and the signal sources are in phase and 180° out of phase, respectively. In both cases (Figs. 4a and 4b), the E field distributions are identical and independent of the phase of the signal sources. The results are summarized in Table 2, where the percentage of volume of the muscle where the E field is greater than a certain value has been computed and tabulated. It is observed that when the input power to one waveguide is 1 W and to the other is 250 mW, a larger volume of the muscle is exposed to higher E field. Measuring the temperature in Tyrode at the location of the muscle (muscle not present) for MMW exposure revealed that continuous wave (CW) 94 GHz E fields raised the temperature of the Tyrode solution in the muscle location by 3-4 °C, even with only a single waveguide. The magnitude of the increase was sufficient to exceed the threshold temperature (38°C) above which muscle contractile force starts to decrease. These results are shown in Fig. 5 which compares

muscle contractile force in response to 60-Hz stimulation under three conditions: waveguides not present (Fig. 5a) to show reproducibility of repeated electric stimulations, waveguides present with no field being delivered but all attendant MMW equipment on (Fig. 5b) to show the lack of effect of the MMW delivery system on measurements, and in the presence of CW exposure at 94 GHz and 250 mW with only one waveguide (Fig. 5c) to verify penetration of the field by measuring the rise in temperature.

To avoid heating during experiments, the input power was pulse modulated while maintaining the maximum available power in the two waveguides (1 W and 250 mW respectively). This reduced the average power delivered to the muscle while maintaining a high peak power. The values of pulse width (PW) and pulse repetition rates (PRR) that do not cause the temperature at the location of the muscle to rise 0.2°C above the physiological value of 35 °C, for each pre-determined set of MMW exposure parameters, were obtained by measuring the temperature at the location of the muscle before each experiment (Table 3). In the experiments, only these PWs and PRRs were used, ensuring that only non-thermal bioeffects were studied.

TABLE 3
MMW PARAMETERS FOR NON-THERMAL
EXPOSURES

Pulse Width (μ s)	Pulse Repetition Rate (kHz)	
	Waveguide input powers 250 mW and 250 mW	Waveguide input powers 250 mW and 1 W
0.02	1500	500
0.1	300	100
1	30	10
10	3	1
100	0.3	0.1
1000	0.03	0.01

Experimental

Table 4 summarizes MMW exposure parameters used in our experiments, the peak electric field magnitude computed using SEMCAD at the location of the muscle, and the number of experiments and exposures for each MMW parameter paradigm.

Table 4: Summary of MMW exposure parameters used in experiments

Experiment Type	MMW exposure	Frequency (GHz) Left waveguide, right waveguide	PW ¹ (μs)	Peak E-field Magnitude ² (kV/m)	No. of Experiments	No. of Exposures
Series 1	Pulse modulation	94, 94	1k, 10k	0.95	6	12
		94, 94	0.1 – 10k	1.5	8	16
		88.7, 97.4	1	1.5	3	6
		95.4, 91.7	1	1.5	1	2
Series 2	Low magnitude CW	94, 94		0.011, 0.034	2	8
	Frequency sweep	88-96, 88-96	100k, 150k	0.8	5	20
		88-96, 88-96	10 – 100k	1.5	14	56
	100 Hz frequency offset	93.00000010-93.000000114, 93	10, 10k	1.8	22	88
	High magnitude with pulse modulation	94, 94	1	2.1	1	4
		94, 94	1	2.6	1	4
		94, 94	0.5 – 10k	3.0	7	28
¹ Pulse repetition rate was set to deliver highest E-field magnitude without allowing heating.						
² Peak E-field in the muscle determined from numerical modeling using SEMCAD						

Fig. 6a illustrates the results of a typical control fatiguing experiment. Four fatigue profiles of a muscle were obtained during a sham exposure experiment. The muscle was fatigued for 2 minutes and allowed to rest for 40 minutes. This procedure was repeated three more times to obtain a total of four fatigue profiles. The profiles are normalized to the first contractile force. This result shows that the profiles are almost identical except the first one. This happened frequently throughout experiments, thus the first fatigue profile was ignored from the analysis for all the experiments. The second fatigue profile (control) is compared with the other two exposure profiles (Figs. 6b and 6c) showing that the third and fourth fatigue profiles are almost identical to the control profile. This ensures that the muscle fatigued multiple times can produce similar fatigue profiles, hence, in actual MMW exposure experiments, the muscle was exposed to MMW fields during the third and the fourth fatigue profiles and the results were compared with that of the second fatigue profile to identify any changes in the contractile force due to MMW exposure.

Fatigue experiments (series 1 as described in Section D of the Materials, Methods and

Design section) produced up to four consistent force profiles for the same muscle, but these experiments showed no effect on contractile force during or immediately after MMW exposure.

Low magnitude CW exposure experiments (series 2 as described in Section D of the Materials, Methods and Design section) did not show any effect on contractile force measurements. In addition, higher magnitude E-fields (0.8, 1.5, 1.8 kV/m) and a variety of pulse modulation schemes had no effect on contractile force.

However, at peak E-field magnitudes of 2.6-3 kV/m, a consistent and reproducible drop in contractile force was observed for a minimum pulse width of 0.75 μ s. This drop was repeated with each successive MMW exposure to the same muscle, and the muscle never recovered its pre-exposure strength (Fig. 7). At 2.6 kV/m, this effect was slight (about 5%), and it was more pronounced (about 10%) at 3.0 kV/m field intensity. This indicates that a field magnitude of 2.6 kV/m could be a threshold value for inducing this effect. Conventional heating of the muscle shows [12] that at 38°C there is a drop in force of approximately 15%, and that at 41°C this drop is approximately 45%, but under these conditions the muscle recovers its pre-heating strength as soon as it is returned to 35°C. The fact that the observed exposure effect is irreversible differentiates it from a conventional heating effect.

Summary and Conclusions

A W-band MMW exposure system to conduct contractile force measurements on muscle has been successfully designed and characterized. The system incorporates careful measurement and monitoring of temperature to ensure that there is no temperature increase from the physiological value of 35°C in the region of the muscle. The system is completely automated via LabVIEW programs and can handle different MMW exposure protocols with minimum interference by the operator. The exposure system has been used for conducting contractile force experiments with FDB muscle with the goal of mitigating and/or decreasing skeletal muscle fatigue.

Our results show that pulse modulation did not have a significant effect on force production in skeletal muscle. The only factor which caused a change in force production was E-field magnitude. A threshold magnitude of 2.6 kV/m with minimum pulse width 0.75 μ s produced an irreversible reduction of contractile force production. To the best that we could determine, there was no increase in temperature. However, a heating effect could not be ruled out because there could be MMW-induced heating in the muscle that could not be detected with our equipment. Future work is aimed at verifying the non-thermal nature of this effect and possible mechanisms.

Publications

Part of this research will be published as a paper in the proceedings of the International Microwave Symposium (IMS) to be held in Baltimore, MD in June 2011. Part of this research has been presented in the Bioelectromagnetics conference held in Seoul, S. Korea in June 2010 and will be presented in the Bioelectromagnetics conference to be held in Halifax, Canada in June 2011.

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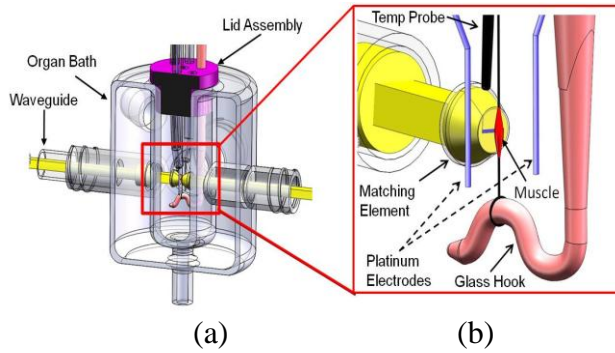


Fig. 1: SolidWorks model of the organ bath showing (a) a cross sectional view with two identical waveguides and (b) a magnified view inside the organ bath (red box) showing placement of the muscle symmetrically between the two platinum electrodes used for electrical stimulation (only one waveguide is shown for clarity).

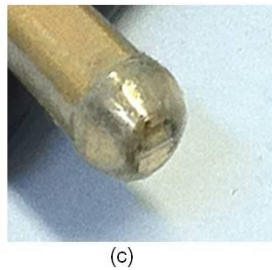
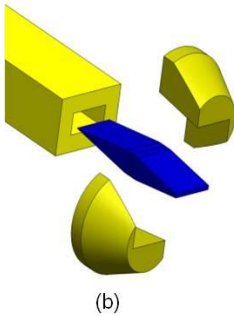
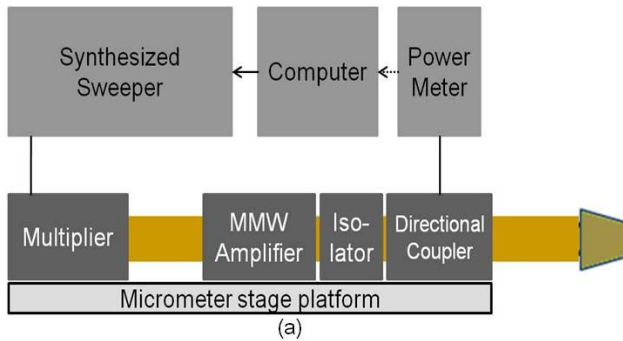


Fig. 2: Schematic of (a) the MMW instrumentation (b) tapered matching element, (c) photograph of the assembled matching element.

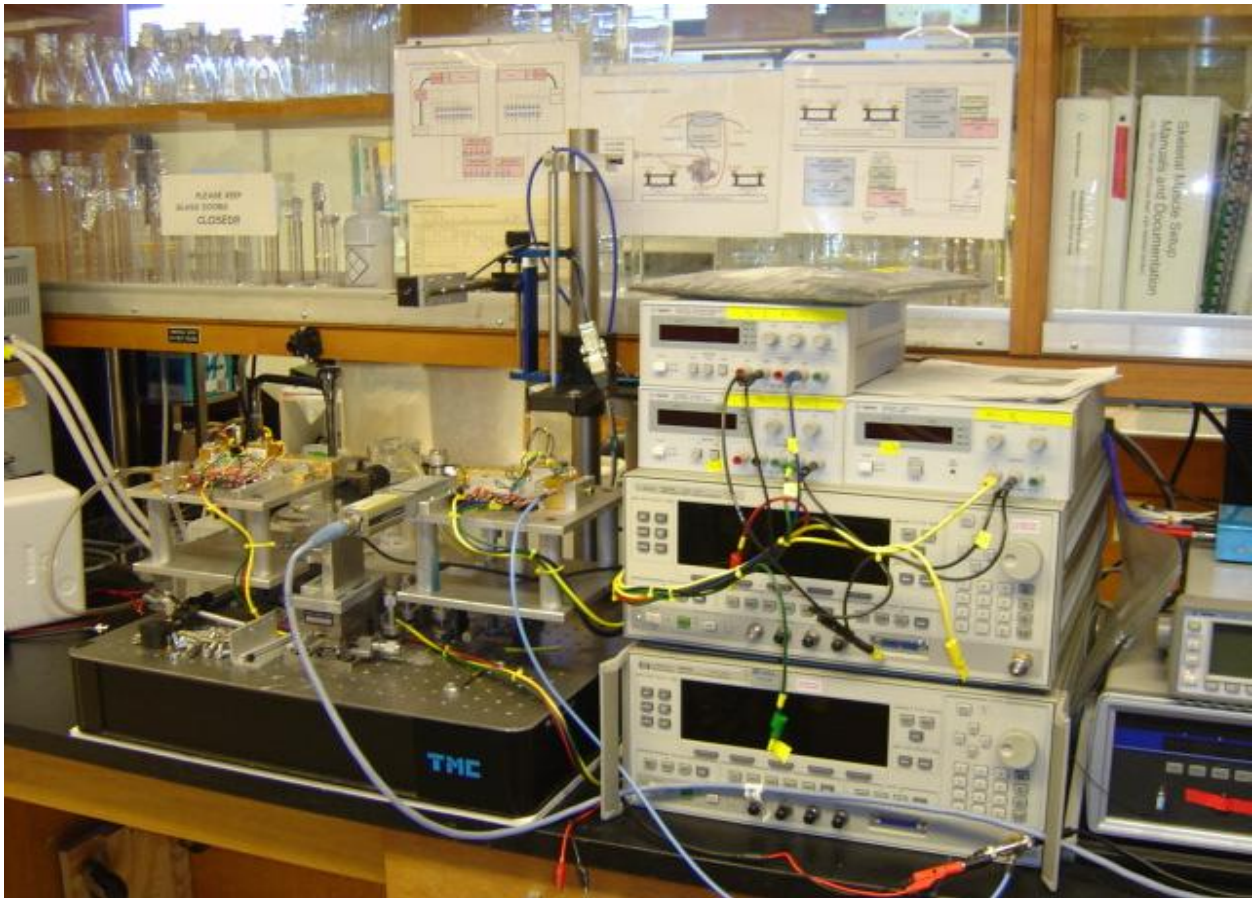


Fig. 2d: Photograph of the experimental apparatus showing the breadboard with all MMW components and the MMW signal sources.

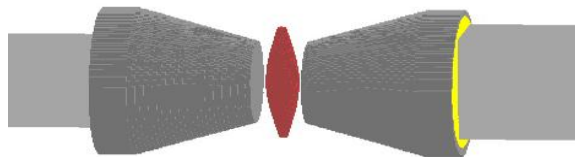
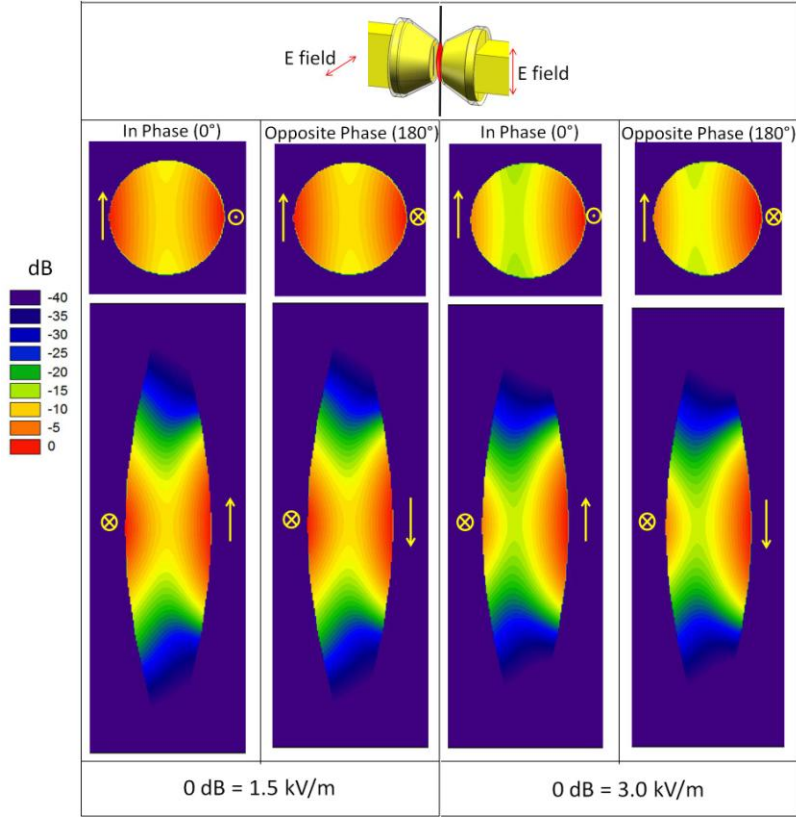


Fig. 3: The 3D FDTD mesh of the MMW exposure system generated in SEMCAD, showing the muscle and two waveguides terminated with matching elements. The two platinum stimulating electrodes and Tyrode solution are not shown for clarity.



(a)

(b)

Fig. 4: Electric field distribution in the region containing the muscle, computed using SEMCAD when (a) input power to each of the waveguides is 250 mW and the signals are in phase (left) and out of phase by 180° (right), (b) input power to one waveguide is 250 mW and to the other is 1 W and the signals are in phase (left) and out of phase by 180° (right). In both cases the directions of the TE₁₀ mode electric fields in the two waveguides are perpendicular to each other.

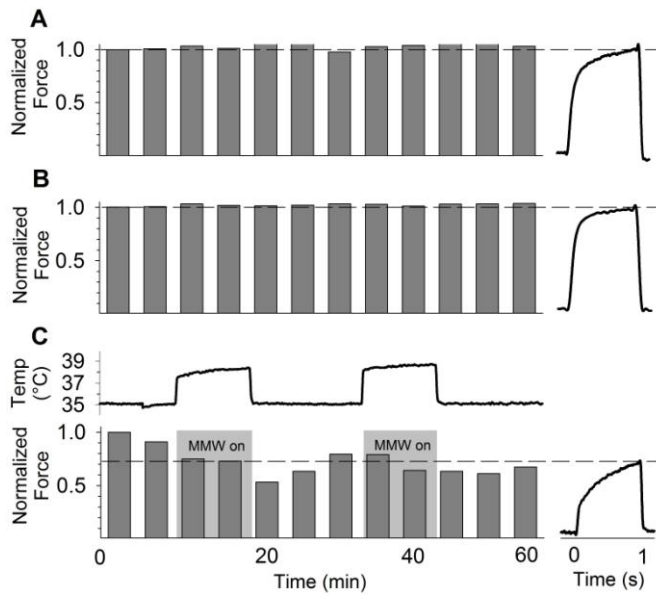
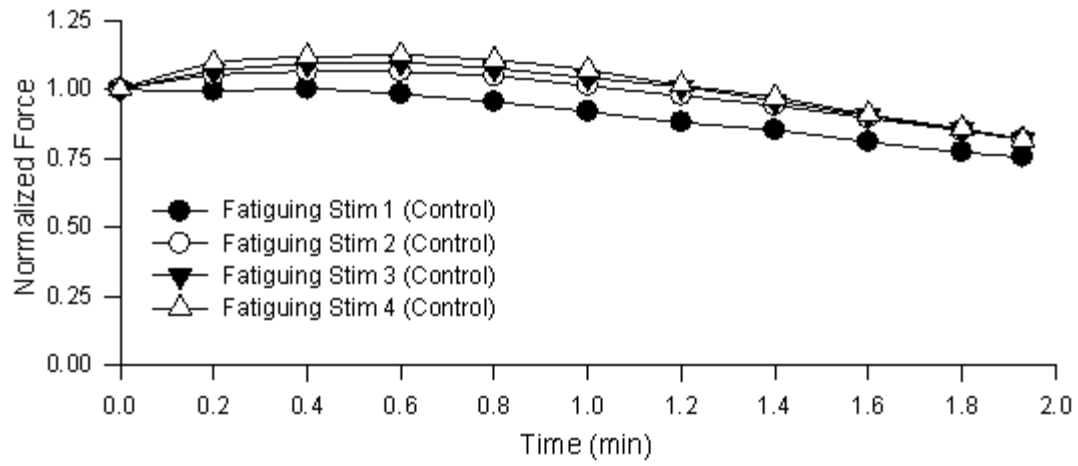
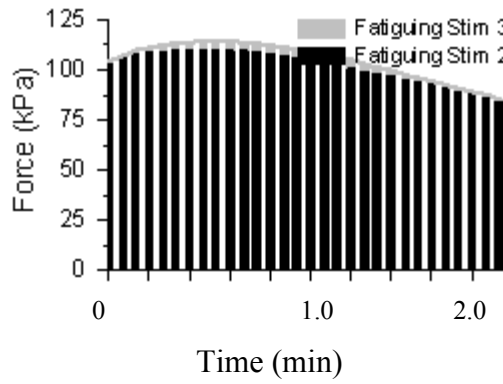


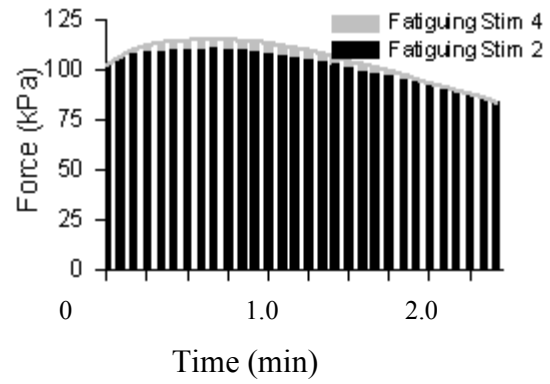
Fig 5: Contractile force measured every five minutes over a 60-minute interval. (A) Control. (B) Sham exposure where the MMW generating equipment is on. (C) MMW exposure (CW at 250 mW). Each value represents force generated by 60-Hz stimulation and is normalized to the first force measurement. Traces at the right are the actual force profiles for the 4th measurement in each series. In (C), the rise in temperature (3-4°C) caused by the MMW field is shown above the bar graph.



(a)



(b)



(c)

Fig. 6: Typical fatiguing profiles obtained during a sham exposure experiment (all MMW equipment on but no input power into the waveguides). Normalized contractile force vs. time for (a) all four fatiguing stimulation series (b) Fatiguing stimulations 3 and 2 (c) Fatiguing stimulations 4 and 2.

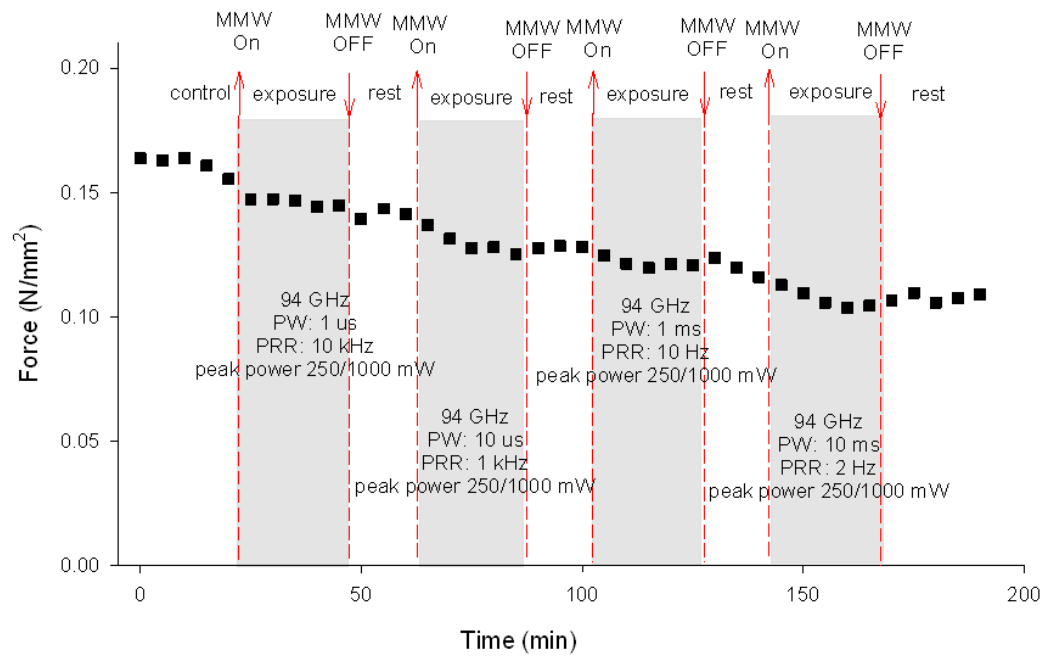


Fig. 7: Typical normalized contractile force vs. time for a MMW exposure experiment with power inputs of 250 mW and 1 W respectively to the two waveguides. The force repeatedly dropped with each successive MMW exposure.